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THE ISOLATION OF ELASTIC TISSUE OF ANIMAL SKIN*

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ABSTRACT

In recent years the behavior of the elastic tissue of animal skin has been under investigation to elucidate its properties. These investigations will be reviewed, and methods of isolation of the elastic tissue from skin and leather will be presented. The isolation methods developed and staining techniques previously developed can be used to determine the modification in the elastic tissue brought about by the various chemicals used in processing skin into leather.

INTRODUCTION

The elastic tissue of animal skin has been studied by many investigators, and a comprehensive review of the occurrence, preparation, and general properties was given by Highberger (1). In this review he mentions that elastic tissue occurs in very small quantities in the skin, the largest amounts being found in the grain layer.

It was reported by Roddy and O'Flaherty (2) that the elastic tissue of calfskin was reduced in amount from that in the fresh skin, but not completely removed, when the stock was processed and finished as chrome-tanned or vegetable-tanned leather. Observations were also made on the appearance of *ligamentum nuchae* (the elastic tissue neck ligament of a calf) which was given the equivalent of a tannery process. Even when the tissue was processed as a particle size obtained by grinding through the finest-mesh screen on the Micro-Wiley Mill, the finely divided elastic tissue particles became hard and harsh when dried from any stage in process, were the consistency of hard wood particles when dried after vegetable tanning, and were similar to glass particles in hardness when chrome-tanned and dried.

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Hoover *et al.* (3) recently have been able to isolate the grain layer of bovine skin and found it to have properties closely related to those of elastic tissue. The yield of their isolated material was essentially the same per unit area of skin for both calfskin and steerhide.

In further work on the network structure of elastin in the grain layer of cattlehide Mellon and Korn (4) showed by horizontal sectioning and chemical fractionation of the sections that the elastin was a three-dimensional network which extended through the thickness of the grain structure. It was shown that the condensed membrane layer obtained was due to the collapse of this network. They further indicated that a maximum concentration of elastin occurs at about one-third the depth of the grain layer and that commercial trypsin was able to dissolve this elastin network from the surface layers. They used a technique whereby the elastin was separated from the skin collagen by extraction with 0.1*N* sodium hydroxide for 16 hours; then the residue was resuspended in fresh alkali for one hour, after which time it was neutralized and centrifuged. The residue was then suspended in water and autoclaved for 2 hours at 23 pounds steam pressure. After it was cooled and centrifuged, the gelatin extract was decanted, and total nitrogen was determined on the extract and the residue. The elastin nitrogen averaged approximately 2% of the total nitrogen for the epidermal area of the hide.

In a private communication (5) it was requested that limed, unhaired calfskin which was treated with a high trypsin bate be examined in cross section to see if the stock so treated had all of the elastic tissue removed by the treatment. The cross sections of the stock when stained with Weigert's Elastic Tissue Stain showed only fragments of elastic tissue in the blood vessels in the center of the corium, which would indicate that the treatment had removed most of the elastic tissue. However, there was the possibility that the elastic tissue might still be present and its staining characteristics altered. To determine by chemical analyses just how much elastic tissue was present in limed stock before bating and the effect of using strong enzyme action to remove the elastic tissue, the present study was undertaken.

Tancous (6), using her previously developed fractionation technique for determining protein fractions of animal skin, has shown that the purifying action of the normal beamhouse procedure will remove practically all of the nonfibrous protein material from calfskin. She obtained a fraction consisting of elastin and hair-root debris amounting to only 2.2% of the total skin protein. Stubbings (7), in attempting to separate these two components, after using a similar fractionation scheme, found that the use of strong sodium sulfide to dissolve the hair residue also dissolved about 15% of the elastin.

Attempts have been made in this laboratory to purify the elastin-keratin residue by dissolving the elastin component by means of enzymes. However, blood vessels are extremely resistant to enzyme action and will always leave a residue of elastin under any practical tannery conditions. In addition, difficulties are encountered in rehydrating the dried residues.

In the present study a measure of removal of elastin from limed, unhaired, and fleshed stock by means of enzyme action was desired. The hair-root residue in any small area of a skin is quite constant as shown by microscopic examination of cross sections. By relating the weight of elastin-keratin residue to the dry weight of skin it is thus possible to follow the reduction in percentage weight of residue brought about by various treatments with the assurance that such reduction is due mainly to elastin removal.

To indicate what occurs to the elastic tissue of animal skin during process it is possible to follow the changes by microscopic examination of stained sections of the skin or by dissolving the collagenous tissue and determining the amount of elastic-tissue residue. For the purpose of orientation the following section on the histology of skin, both untreated and treated, is given.

MICROSCOPIC EXAMINATION

When cross sections or sections cut in a plane parallel to the hair side are stained with Weigert's Elastic Tissue Stain, the following photomicrographs can be made of the elastic tissue of the skin. In viewing these sections it is to be kept in mind that the elastic tissue is present as a network surrounding the hair follicles in the epidermal area and as part of the arteries found throughout the skin. Such arteries are responsible for the so-called veins which are unsightly on the finished leather when prominent.

In Fig. 1 a cross section of fresh calfskin stained with Weigert's Elastic Tissue Stain is shown. In this cross section the elastic tissue is seen mainly in the epidermal area and on the flesh side. It is located in the epidermal area as a basketlike network which extends from the epidermis to the sweat

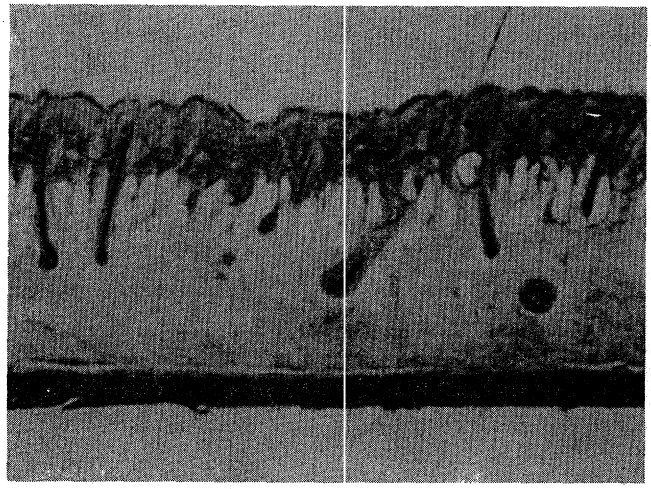

FIGURE 1.—Cross section of fresh calfskin stained with Weigert's Elastic Tissue Stain.

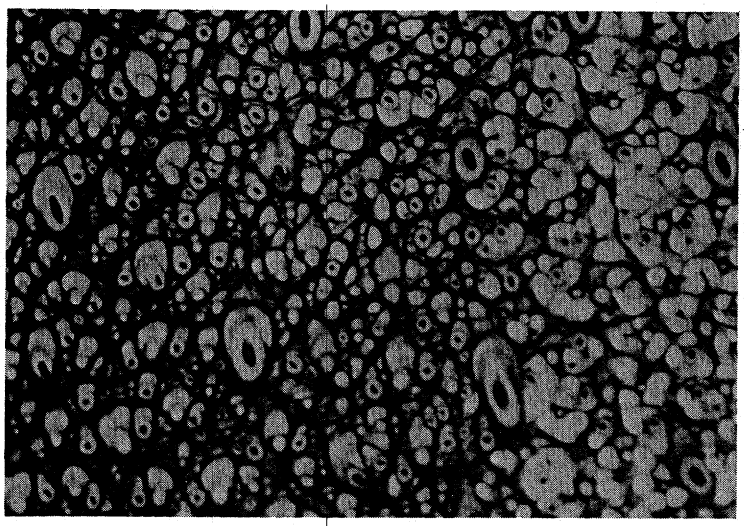

FIGURE 2.—Section cut in horizontal plane of fresh calfskin stained with Weigert's Elastic Tissue Stain.

FIGURE 3.—Cross section of the epidermal area of fresh calfskin stained with Weigert's Elastic Tissue Stain.

glands surrounding each hair pocket. Figure 2 is a section cut in a horizontal plane with the portion on the left side showing the network of the elastic tissue surrounding each hair pocket and the right-hand portion the sweat glands. The only elastic tissue in this area is in the arteries running between the sweat glands. For a more detailed picture of the elastic tissue in the epidermal area Fig. 3 is shown. The elastic tissue is more dense in the oil gland region and shows finer fibers as the tissue attaches to the epidermis. At the top of the oil glands are three arteries cut in cross section containing elastic tissue as part of their walls.

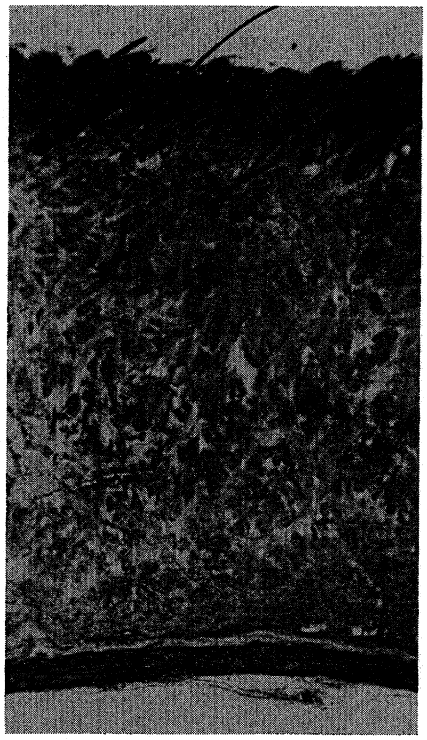

FIGURE 4.—Cross section of fresh steerhide stained with Weigert's Elastic Tissue Stain.

In a cross section of fresh steerhide as shown in Fig. 4 the elastic tissue is similar to that seen in calfskin, being located in the epidermal area and on the flesh side when stained with Weigert's Elastic Tissue Stain. At higher magnification the elastic tissue of steerhide appears as shown in Fig. 5. As in the calfskin most of the elastic tissue is located in the region of the oil glands with little elastic tissue being present below the top of the sweat glands. The arteries present also have the elastic tissue as part of their walls.

When a piece of fresh calfskin is placed in the autoclave for 4 hours at 15 pounds pressure, the collagen is digested, and the epidermis, its appendages, and the elastic tissue remain behind as a residue. A picture of such treated stock in cross section is shown in Fig. 6. The epidermis swells as a result of such treatment but still retains most of its original structure. The hair roots also are altered but still present. The elastic tissue tends to contract but is not greatly altered.

After the bating operation the residue remaining appears as a sheet of tissue as shown in Fig. 7. This tissue has been stained with Weigert's Elastic Tissue Stain; at this magnification the fibers are not easily identified, but under higher magnification the fibers can be seen. A stained cross section of the sheet shows the elastic tissue fibers to be present but not as receptive to the stain as obtained with the fresh skin in Fig. 6.

FIGURE 5.—Cross section of the epidermal area of fresh steerhide stained with Weigert's Elastic Tissue Stain.

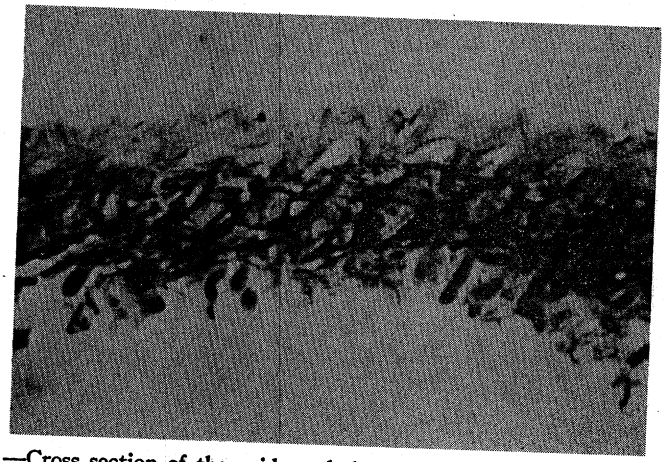

FIGURE 6.—Cross section of the epidermal sheet remaining after being autoclaved for 4 hours at 15 pounds pressure and then stained with Weigert's Elastic Tissue Stain.

When a horizontal section is cut of the epidermal area of dyed and finished calfskin leather and the section decolorized and then stained with Weigert's Elastic Tissue Stain, the picture shown in Fig. 8 is obtained. The elastic

tissue has been reduced in amount, but broken fibers still remain. Examination of these fibers in the finished leather at high magnification shows them to be broken into a series of fine particles rather than as the continuous fibers which are seen in the fresh skin.

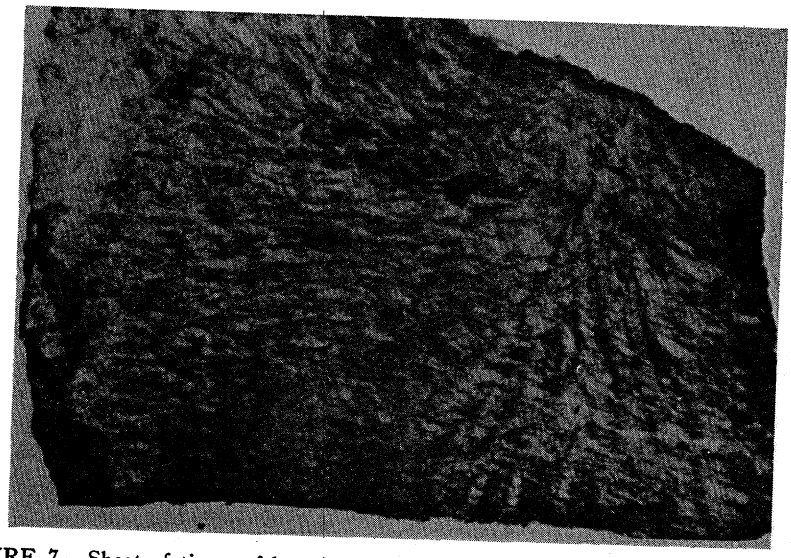

FIGURE 7.—Sheet of tissue of bated calfskin which had been autoclaved at 15 pounds pressure and then stained with Weigert's Elastic Tissue Stain.

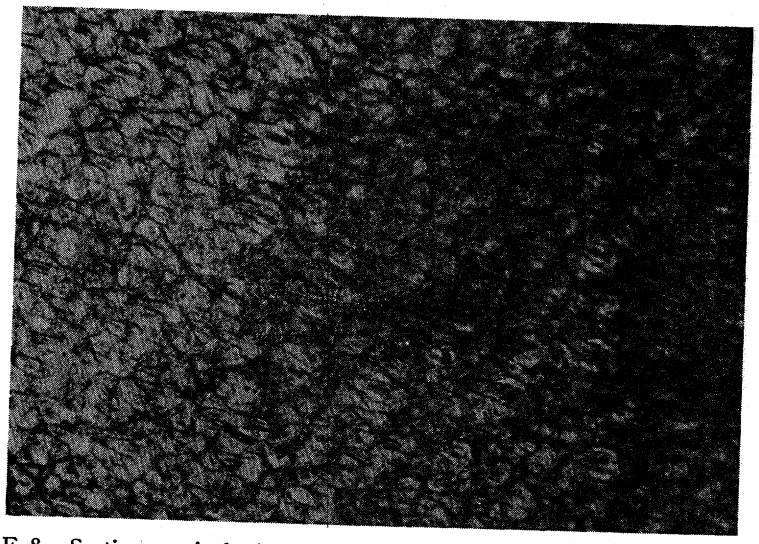

FIGURE 8.—Section cut in horizontal plane of calfskin leather stained with Weigert's Elastic Tissue Stain.

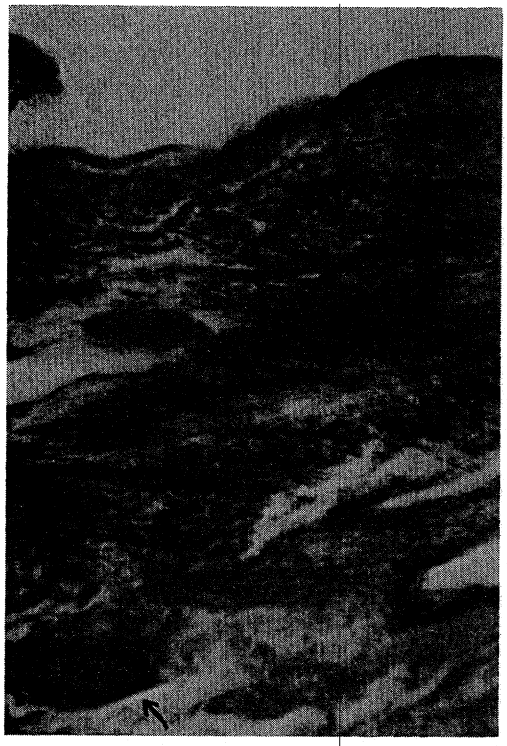

FIGURE 9.—Cross section of the epidermal area of chrome-tanned calfskin stained with Weigert's Elastic Tissue Stain.

In Fig. 9 the elastic tissue in the blood vessels in the epidermal area of chrome-tanned leather is shown. These are the smaller blood vessels near the grain surface and the larger blood vessel near the base of the hair roots which are readily traced by staining with Weigert's Elastic Tissue Stain.

The foregoing figures indicate that elastic tissue is altered by processing and that some of the tissue may be removed but that the residue is still very demonstrable. As indicated by the work of Tancous (6) and Stubbings (7) the normal beamhouse procedures remove practically all the nonfibrous proteins from skin. Hydrolysis of the collagen from the skin results in the fraction of elastin and hair-root debris. As the hair-root debris was reasonably uniform over the skins selected for the present work, the elastin could be calculated using two procedures developed. One procedure was for limed, unhaired skins, and the other procedure was for stock processed in the tannery to the "chrome in the blue" state. For the limed, unhaired stock the procedure selected has as a major feature the deliming of the residue, which is not necessary for the tanned stock as the results obtained from the two procedures will show.

LABORATORY PROCEDURES FOR DETERMINATION OF ELASTIN-KERATIN RESIDUE

Procedure I—(Elastin-keratin residue in limed and bated stock)

Preparation of lime stock.—Cured calfskin stock was fleshed and soaked in water for 16 hours at room temperature in the proportion of 4 parts of water to 1 of cured skin. A second soak in fresh water in the same proportions for

a period of 4 hours was then given. The skin was limed with straight lime solution for 7 days using a 4-to-1 ratio of water to cured-skin weight and 12% hydrated lime on the cured weight. The stock was then unhaired, scudded thoroughly, and fleshed. Blocks of 4 specimens, each specimen 2¾" x 2¾", were cut from over the whole skin to furnish 3 test lots and one control lot.

Removal of elastin.—The stock was surface-delimed with hydrochloric acid, then completely delimed with boric acid at 75°F. Controlled conditions were established using large bottles immersed in a bath at 75°F. and rotating at 45 rpm. Then 1% $NaHCO_3$ on the white weight was added, and the temperature was raised to 90°F. The elastin-removing enzyme (powder* from a whole raw pancreas, activated, desiccated, and defatted) was added, and bating was continued for the required time. The squares were then blotted on towels, wrapped in wax paper and aluminum foil, and placed in a freezer to stop enzyme action.

Digestion of soluble hide components.—After being defrosted the specimen squares were individually pressed in a Carver press at 10,000 pounds ram pressure to remove most of the water. They were then dried overnight in a vacuum oven at 80°C. and 22 inches of mercury, cooled in a desiccator, and weighed.

The dried squares were placed in separate 400-ml. beakers, and sufficient distilled water was added to yield approximately a 3% gelatin solution. The beakers were covered and placed in an autoclave for 4 hours at 15 pounds steam pressure. After this the hot solutions were decanted through previously dried and weighed coarse-fritted glass crucibles. The residues in the beakers were treated with hot 0.5*N* HCl to remove calcium, then transferred to the crucibles with hot water. The crucibles were then washed alternately with hot acid and hot water until all lime and acid were removed from the residues.

The washed crucibles were placed in Soxhlet equipment, and the grease in the residues was removed by extraction with acetone for 4 hours. The crucibles were then air-dried and finally placed in a desiccator overnight to dry to constant weight. The percent residue of the dried squares was calculated. Comparison of the percent residues of treated lots with that of the control gave a measurement of degree of removal of elastin.

Procedure II—(Elastin-keratin residue in leather in the blue)

Twenty-seven specimen squares were cut from matching positions from right and left sides of the three skins being examined. After being blotted with filter paper each square was weighed to establish reference weights and then placed in Petri dishes. Fifty ml. of distilled water was added to each dish, and the dishes containing the specimens were placed in an autoclave

*Viokase powder P-145-D, produced by the Viobin Corporation, Monticello, Ill.

for 4 hours at 15 pounds steam pressure. The dishes were then removed from the autoclave, the solutions were poured off the specimens, and the specimens were washed in distilled water and then soaked in water overnight.

After soaking in water overnight the specimens were blotted with filter paper to a condition where they no longer showed a water mark on the filter paper. The specimens were then weighed, and the percent elastic-tissue residue was calculated.

A portion of each residue after soaking overnight was stained with Weigert's Elastic Tissue Stain to demonstrate the elastic tissue in the residue. This demonstrated both the elastic tissue in the basketlike network of the epidermal area and the elastic tissue in the arteries.

EXPERIMENTAL RESULTS

Table I shows illustrative data obtained from two limed calfskins.

TABLE I

PERCENT ELASTIC TISSUE AND KERATIN REMAINING IN CALFSKIN AFTER BATING

(*Average of 20 values*)

	1st Skin		2nd Skin	
	Control	0.01% Enzyme	Control	0.03% Enzyme
Percent residue	2.21	1.55	2.78	0.38
Standard deviation	0.41	0.33	0.43	0.11
Percent decrease in residue		30		86

Examination of the values for the percent residue in the two calfskins before bating, listed as controls in Table I, shows that the first calfskin contains less elastic tissue then the second calfskin. When portions of the two bated calfskin residues were stained and examined microscopically, the elastic tissue in the residues was still present but broken into fragments rather than as a continuous sheet. There was very little residue from the calfskin specimens treated with the 0.03% enzyme.

Although the standard deviations are fairly high, representing as they do the differences in blocks over the whole skin and including effects of thickness differences and possibly keratin residue differences in addition to experimental error, statistically they permit a 95% assurance that even an 11% decrease in residue would have significance for the first skin and 8% for the second skin.

In Table II the data obtained from the analysis of the three skins in the blue are arranged to show symmetry between sides, differences between rows parallel to the backbone, and a comparison between the skins. The stained

residues from each calfskin were examined microscopically, and it was observed that the arteries were similar in distribution and size on each side of the calfskins. The veiny calfskins showed more pronounced and less diffuse arteries present in the residues than those observed in the non-veiny calfskin.

It is obvious that the veiny leather has more elastic tissue residue than the normal leather. Although a somewhat less precise technique was used in obtaining the values shown than in the case of the limed skins, it is informative to know that such a rapid method can be used to produce useful results.

TABLE II

PERCENT ELASTIC TISSUE AND KERATIN
IN NON-VEINY AND VEINY CALFSKIN LEATHER

(*Average of 27 values per side*)

	Non-Veiny Skin		Veiny Skin No. 1		Veiny Skin No. 2	
	Left Side	Right Side	Left Side	Right Side	Left Side	Right Side
Percent residue	1.91	2.19	2.74	2.79	2.77	2.94
Standard deviation	0.25	0.18	0.27	0.27	0.25	0.34

CONCLUSIONS

By microscopic examination of cross sections stained with an elastic-tissue stain and by the two methods developed for isolating the elastic-tissue residue of animal skin it is possible to determine the influence of processing operations on the elastic tissue. In regular calfskin processing there is a reduction in the amount of elastic tissue from that found in salt-cured calfskin.

When sufficient enzyme specific for breaking down elastic tissue is used, it is possible to remove almost all of the elastic tissue from the skin or hide. In the present work under the test conditions used it was possible to remove 30% and 86% of the elastic tissue in the bating operation by using an enzyme powder from whole raw pancreas, which was activated, desiccated, and defatted.

A comparison of non-veiny and veiny calfskins showed that the veiny calfskins contained more elastic tissue. The comparison made also established that there are similar amounts of the elastic tissue on each side of the calfskin, showing that symmetry occurs between sides of a given skin.

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DISCUSSION

DR. EDWARD F. MELLON (Eastern Regional Research Laboratory): We are indebted to Mr. Roddy for an excellent demonstration of the ability of histological techniques and chemical determinations to show us the location of elastic tissue and the amount of elastic tissue present in a hide.

An observation which we had made at the time we demonstrated the elastic-tissue network in the hide confirms very well the histological demonstration which you saw this morning of a high concentration of elastic tissue around the hair follicle and just about midway down the depth of the grain layer.

The slices which we had cut, which were about one-tenth of a millimeter thick, at the grain surface did not show any large concentration right at the hair follicle. But as we reached this medium depth of the grain layer the concentration of elastin was so great that in these small slices you could actually see a bump where the hair follicles were. There was a bump, a ridge around the outside, and the hole where the hair shaft had gone through it. I think this is very good confirmation of the histological pictures which we saw today.

I have one question: In the breakdown of these fibers which we have noted, after you have treated the hide under certain conditions, would you say that this breakdown of the fibers is something that might be progressive? Do you dissolve the outside of the fibers, making them smaller until they finally break through, or do you break off a chunk and leave the middle of the fibers?

MR. RODDY: That is a very nice question.

There has been information and literature in recent years that in staining elastic tissue what you are really doing is staining perhaps a mucoid fraction surrounding the individual elastic-tissue fiber. Some years ago when we worked with the neck ligament and cut it in cross section, the fibers in cross section were stained completely with the Weigert's Elastic Tissue Stain.

Now the question brought up by Dr. Mellon was: Is this elastic tissue gradually broken down? It definitely is. As an example, if we leave it in the autoclave for a period of 6 to 8 hours, we will dissolve much more elastic tissue than if we leave it in for the 2 to 4 hours that we selected in the present work. So there definitely is a breakdown. However, the point I tried to

make in the course of my talk is that the elastic tissue during the course of ordinary tanning is still present in the finished leather and still demonstrable by Weigert's Elastic Tissue Stain; and also we can recover this elastic-tissue residue from calfskin as shown in the case of the chrome stock where we recovered as much as 2% from the non-veiny skin and 2.75% to almost 3% in the case of the veiny skins.

DR. SELIGSBERGER (Quartermaster Research and Engineering Center): Do I understand you right, that keratin is included in the figures in the table? And how much keratin is actually present?

MR. RODDY: I mentioned in the course of the talk, Dr. Seligsberger, that Dr. Stubbings tried to remove this hair-root keratin from the elastic-tissue fraction and in the course of doing that he was able, using strong sulfide, to remove the keratinous material, but he also reduced the elastic tissue. Tancous, in her work, showed the elastic-tissue fraction was 2.2%. On the other hand, the work by Dr. Mellon and coworkers showed that the elastic tissue as a residue based on total nitrogen was somewhere in the neighborhood of 2%. So the elastic-tissue residue is somewhere in that particular region, and the small amount of keratin you would have as a hair-root debris, which is not removed by standard processing, would only be a very small amount apparently.

MILTON BAILEY (U. S. Navy Research & Development Facility): We have had some rather interesting experience with calfskin versus stuffed side leathers so far as water-resistance deterioration is concerned. We found that stuffed leathers would crack across the vamps very rapidly, whereas our calfskin withstood this kind of wear for considerable periods of time. I wonder if the elastic tissue is a major factor in causing this kind of resistance as far as calfskin is concerned.

MR. RODDY: We do not have that information as yet. However, at the present time at Cincinnati we are processing both side upper leather and calfskin leathers to determine exactly what influence this elastic-tissue removal will have upon the finished leathers.

DR. H. B. MERRILL (B. D. Eisendrath Tanning Co.): It seems very remarkable that according to what Mr. Roddy has said, the autoclaving process actually destroys collagen even when it is chrome-tanned, without affecting the elastin. Is that correct, Mr. Roddy?

MR. RODDY: It removes both the collagen and also the chrome-tanning material. The process when used on vegetable-tanned leather will also dissolve the collagen, but the difficulty you run into is that you get polymeriza-

tion of the vegetable tanning materials with the breakdown in the collagen, and, of course, the elastic-tissue residue becomes much harder to separate. I might also point out at this time that if we take fresh skin or hide and use this autoclaving technique, it is possible to remove the collagen and leave the epidermis (reasonably intact with slight swelling of the epidermal cells), the hair, the oil glands, and the elastic tissue as a residue.

DR. JOSEPH NAGHSKI (Eastern Utilization Research & Development Division): Workers at the American Meat Institute Foundation found when they studied the elastic tissue in meat that the enzymes digested out the center portion of the elastin fibers leaving a ghostlike shell. Did you observe anything like that in the skin tissue as contrasted with the meat?

MR. RODDY: No, we did not. I brought up the point a while ago that when we took the ligament and cut it into cross section we did not come up with the shell-like idea. You can stain the fiber completely through even in the case of finished leather.

DR. ANDREW SALAMATOV (Barrett & Co. Inc.): I would like to pursue the sulfide a little further. You said strong sulfide removes 15%. What is meant by "strong"?

The second part of my question is: If it dissolves 15% of skin, what happens to the rest of it? Maybe it is broken down by sulfide treatment.

MR. RODDY: The work which I was quoting was that of Dr. Stubbings, and of course, he was removing the keratin residue from the elastic tissue which he had isolated, and that is where the 15% breakdown of keratin occurred.

It is possible, as an example (I mentioned it previously), just by water to ultimately digest the elastic tissue. Therefore acid or alkali will also break it down. The point that is made is this: It happens to be apparently much more resistant tissue in an aqueous system than the collagen, and therefore you can make a separation in a manner in which we have done—from fresh skin all the way through processing and on tanned stock. The only exception which I mentioned was in the case of the vegetable-tanned stock. We had difficulty there. On the other hand, with care and proper removal of the water from the stock during the course of autoclaving, it is also possible to obtain a residue from vegetable-tanned stock. It is a little more tedious to do, but it can also be obtained.

DR. HAROLD G. TURLEY (Rohm & Haas): I do hope the impression is not given by the speakers here that all this work was original. I suppose 35 years ago it was a controversial question in this Association. I do hope that due

credit will be given to Dr. John Arthur Wilson for the demonstration of elastin and the way it behaved in tannery treatments, and also to Dr. Hollander who proved at that time in practical leathermaking that elastin is broken down and not necessarily removed.

However, I do find interesting and original this demonstration of the greater amount of elastin in veiny hides. But I would like you to show, in connection with the technique, that there is some correction applied there with regard to residues. I am wondering if you have done any work with pure isolated elastin itself, putting it through the different techniques, and whether you have some idea of the recovery.

MR. RODDY: We are giving full recognition to the work done by John Arthur Wilson, Hollander, and previous investigators.

Our major issue here, of course, is to show that it is possible by chemical analysis or a chemical technique to isolate the elastic tissue.

I appreciate, as you do, Dr. Turley, the work that has gone on before, not only by John Arthur Wilson, but also by Dr. Merrill who is in the audience, and who has used a similar technique for enzyme removal of the elastic tissue of calfskin, and I would like to go on record to say that is the case.

In regard to using the pure elastic tissue, some years back when we presented information on using the elastic tissue as a biological source from the neck ligament, it was argued as to whether it would resemble the elastic tissue in animal skin.

I might point out that in the course of doing this work in Cincinnati we had a man by the name of Earl Chia, from Formosa, visit our laboratory. Upon seeing the elastic tissue removed from skin and hide, and the ligament put into the autoclave for digestion purposes, he was very curious. I explained what we were doing. And of course he predates anyone else from the standpoint of the record of using a technique of this nature. He says that it was handed down in his family for generations. The pressure cooker was used to digest ligament and collagen to break it down to protein of a soluble nature and the right size so that it could be ingested by the family. So it predates everything.

As I say, then, Dr. Turley, I would like to give recognition to the gentlemen mentioned and to anyone else who has been concerned with this type of work.

a period of 4 hours was then given. The skin was limed with straight lime solution for 7 days using a 4-to-1 ratio of water to cured-skin weight and 12% hydrated lime on the cured weight. The stock was then unhaired, scudded thoroughly, and fleshed. Blocks of 4 specimens, each specimen 2¾" x 2¾", were cut from over the whole skin to furnish 3 test lots and one control lot.

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The washed crucibles were placed in Soxhlet equipment, and the grease in the residues was removed by extraction with acetone for 4 hours. The crucibles were then air-dried and finally placed in a desiccator overnight to dry to constant weight. The percent residue of the dried squares was calculated. Comparison of the percent residues of treated lots with that of the control gave a measurement of degree of removal of elastin.

Procedure II—(Elastin-keratin residue in leather in the blue)

Twenty-seven specimen squares were cut from matching positions from right and left sides of the three skins being examined. After being blotted with filter paper each square was weighed to establish reference weights and then placed in Petri dishes. Fifty ml. of distilled water was added to each dish, and the dishes containing the specimens were placed in an autoclave

*Viokase powder P-145-D, produced by the Viobin Corporation, Monticello, Ill.

for 4 hours at 15 pounds steam pressure. The dishes were then removed from the autoclave, the solutions were poured off the specimens, and the specimens were washed in distilled water and then soaked in water overnight.

After soaking in water overnight the specimens were blotted with filter paper to a condition where they no longer showed a water mark on the filter paper. The specimens were then weighed, and the percent elastic-tissue residue was calculated.

A portion of each residue after soaking overnight was stained with Weigert's Elastic Tissue Stain to demonstrate the elastic tissue in the residue. This demonstrated both the elastic tissue in the basketlike network of the epidermal area and the elastic tissue in the arteries.

EXPERIMENTAL RESULTS

Table I shows illustrative data obtained from two limed calfskins.

TABLE I

PERCENT ELASTIC TISSUE AND KERATIN REMAINING IN CALFSKIN AFTER BATING

(*Average of 20 values*)

	1st Skin		2nd Skin	
	Control	0.01% Enzyme	Control	0.03% Enzyme
Percent residue	2.21	1.55	2.78	0.38
Standard deviation	0.41	0.33	0.43	0.11
Percent decrease in residue		30		86

Examination of the values for the percent residue in the two calfskins before bating, listed as controls in Table I, shows that the first calfskin contains less elastic tissue then the second calfskin. When portions of the two bated calfskin residues were stained and examined microscopically, the elastic tissue in the residues was still present but broken into fragments rather than as a continuous sheet. There was very little residue from the calfskin specimens treated with the 0.03% enzyme.

Although the standard deviations are fairly high, representing as they do the differences in blocks over the whole skin and including effects of thickness differences and possibly keratin residue differences in addition to experimental error, statistically they permit a 95% assurance that even an 11% decrease in residue would have significance for the first skin and 8% for the second skin.

In Table II the data obtained from the analysis of the three skins in the blue are arranged to show symmetry between sides, differences between rows parallel to the backbone, and a comparison between the skins. The stained

residues from each calfskin were examined microscopically, and it was observed that the arteries were similar in distribution and size on each side of the calfskins. The veiny calfskins showed more pronounced and less diffuse arteries present in the residues than those observed in the non-veiny calfskin.

It is obvious that the veiny leather has more elastic tissue residue than the normal leather. Although a somewhat less precise technique was used in obtaining the values shown than in the case of the limed skins, it is informative to know that such a rapid method can be used to produce useful results.

TABLE II

PERCENT ELASTIC TISSUE AND KERATIN
IN NON-VEINY AND VEINY CALFSKIN LEATHER

(*Average of 27 values per side*)

	Non-Veiny Skin		Veiny Skin No. 1		Veiny Skin No. 2	
	Left Side	Right Side	Left Side	Right Side	Left Side	Right Side
Percent residue	1.91	2.19	2.74	2.79	2.77	2.94
Standard deviation	0.25	0.18	0.27	0.27	0.25	0.34

CONCLUSIONS

By microscopic examination of cross sections stained with an elastic-tissue stain and by the two methods developed for isolating the elastic-tissue residue of animal skin it is possible to determine the influence of processing operations on the elastic tissue. In regular calfskin processing there is a reduction in the amount of elastic tissue from that found in salt-cured calfskin.

When sufficient enzyme specific for breaking down elastic tissue is used, it is possible to remove almost all of the elastic tissue from the skin or hide. In the present work under the test conditions used it was possible to remove 30% and 86% of the elastic tissue in the bating operation by using an enzyme powder from whole raw pancreas, which was activated, desiccated, and defatted.

A comparison of non-veiny and veiny calfskins showed that the veiny calfskins contained more elastic tissue. The comparison made also established that there are similar amounts of the elastic tissue on each side of the calfskin, showing that symmetry occurs between sides of a given skin.

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DISCUSSION

DR. EDWARD F. MELLON (Eastern Regional Research Laboratory): We are indebted to Mr. Roddy for an excellent demonstration of the ability of histological techniques and chemical determinations to show us the location of elastic tissue and the amount of elastic tissue present in a hide.

An observation which we had made at the time we demonstrated the elastic-tissue network in the hide confirms very well the histological demonstration which you saw this morning of a high concentration of elastic tissue around the hair follicle and just about midway down the depth of the grain layer.

The slices which we had cut, which were about one-tenth of a millimeter thick, at the grain surface did not show any large concentration right at the hair follicle. But as we reached this medium depth of the grain layer the concentration of elastin was so great that in these small slices you could actually see a bump where the hair follicles were. There was a bump, a ridge around the outside, and the hole where the hair shaft had gone through it. I think this is very good confirmation of the histological pictures which we saw today.

I have one question: In the breakdown of these fibers which we have noted, after you have treated the hide under certain conditions, would you say that this breakdown of the fibers is something that might be progressive? Do you dissolve the outside of the fibers, making them smaller until they finally break through, or do you break off a chunk and leave the middle of the fibers?

MR. RODDY: That is a very nice question.

There has been information and literature in recent years that in staining elastic tissue what you are really doing is staining perhaps a mucoid fraction surrounding the individual elastic-tissue fiber. Some years ago when we worked with the neck ligament and cut it in cross section, the fibers in cross section were stained completely with the Weigert's Elastic Tissue Stain.

Now the question brought up by Dr. Mellon was: Is this elastic tissue gradually broken down? It definitely is. As an example, if we leave it in the autoclave for a period of 6 to 8 hours, we will dissolve much more elastic tissue than if we leave it in for the 2 to 4 hours that we selected in the present work. So there definitely is a breakdown. However, the point I tried to

make in the course of my talk is that the elastic tissue during the course of ordinary tanning is still present in the finished leather and still demonstrable by Weigert's Elastic Tissue Stain; and also we can recover this elastic-tissue residue from calfskin as shown in the case of the chrome stock where we recovered as much as 2% from the non-veiny skin and 2.75% to almost 3% in the case of the veiny skins.

DR. SELIGSBERGER (Quartermaster Research and Engineering Center): Do I understand you right, that keratin is included in the figures in the table? And how much keratin is actually present?

MR. RODDY: I mentioned in the course of the talk, Dr. Seligsberger, that Dr. Stubbings tried to remove this hair-root keratin from the elastic-tissue fraction and in the course of doing that he was able, using strong sulfide, to remove the keratinous material, but he also reduced the elastic tissue. Tancous, in her work, showed the elastic-tissue fraction was 2.2%. On the other hand, the work by Dr. Mellon and coworkers showed that the elastic tissue as a residue based on total nitrogen was somewhere in the neighborhood of 2%. So the elastic-tissue residue is somewhere in that particular region, and the small amount of keratin you would have as a hair-root debris, which is not removed by standard processing, would only be a very small amount apparently.

MILTON BAILEY (U. S. Navy Research & Development Facility): We have had some rather interesting experience with calfskin versus stuffed side leathers so far as water-resistance deterioration is concerned. We found that stuffed leathers would crack across the vamps very rapidly, whereas our calfskin withstood this kind of wear for considerable periods of time. I wonder if the elastic tissue is a major factor in causing this kind of resistance as far as calfskin is concerned.

MR. RODDY: We do not have that information as yet. However, at the present time at Cincinnati we are processing both side upper leather and calfskin leathers to determine exactly what influence this elastic-tissue removal will have upon the finished leathers.

DR. H. B. MERRILL (B. D. Eisendrath Tanning Co.): It seems very remarkable that according to what Mr. Roddy has said, the autoclaving process actually destroys collagen even when it is chrome-tanned, without affecting the elastin. Is that correct, Mr. Roddy?

MR. RODDY: It removes both the collagen and also the chrome-tanning material. The process when used on vegetable-tanned leather will also dissolve the collagen, but the difficulty you run into is that you get polymeriza-

tion of the vegetable tanning materials with the breakdown in the collagen, and, of course, the elastic-tissue residue becomes much harder to separate. I might also point out at this time that if we take fresh skin or hide and use this autoclaving technique, it is possible to remove the collagen and leave the epidermis (reasonably intact with slight swelling of the epidermal cells), the hair, the oil glands, and the elastic tissue as a residue.

DR. JOSEPH NAGHSKI (Eastern Utilization Research & Development Division): Workers at the American Meat Institute Foundation found when they studied the elastic tissue in meat that the enzymes digested out the center portion of the elastin fibers leaving a ghostlike shell. Did you observe anything like that in the skin tissue as contrasted with the meat?

MR. RODDY: No, we did not. I brought up the point a while ago that when we took the ligament and cut it into cross section we did not come up with the shell-like idea. You can stain the fiber completely through even in the case of finished leather.

DR. ANDREW SALAMATOV (Barrett & Co. Inc.): I would like to pursue the sulfide a little further. You said strong sulfide removes 15%. What is meant by "strong"?

The second part of my question is: If it dissolves 15% of skin, what happens to the rest of it? Maybe it is broken down by sulfide treatment.

MR. RODDY: The work which I was quoting was that of Dr. Stubbings, and of course, he was removing the keratin residue from the elastic tissue which he had isolated, and that is where the 15% breakdown of keratin occurred.

It is possible, as an example (I mentioned it previously), just by water to ultimately digest the elastic tissue. Therefore acid or alkali will also break it down. The point that is made is this: It happens to be apparently much more resistant tissue in an aqueous system than the collagen, and therefore you can make a separation in a manner in which we have done—from fresh skin all the way through processing and on tanned stock. The only exception which I mentioned was in the case of the vegetable-tanned stock. We had difficulty there. On the other hand, with care and proper removal of the water from the stock during the course of autoclaving, it is also possible to obtain a residue from vegetable-tanned stock. It is a little more tedious to do, but it can also be obtained.

DR. HAROLD G. TURLEY (Rohm & Haas): I do hope the impression is not given by the speakers here that all this work was original. I suppose 35 years ago it was a controversial question in this Association. I do hope that due

credit will be given to Dr. John Arthur Wilson for the demonstration of elastin and the way it behaved in tannery treatments, and also to Dr. Hollander who proved at that time in practical leathermaking that elastin is broken down and not necessarily removed.

However, I do find interesting and original this demonstration of the greater amount of elastin in veiny hides. But I would like you to show, in connection with the technique, that there is some correction applied there with regard to residues. I am wondering if you have done any work with pure isolated elastin itself, putting it through the different techniques, and whether you have some idea of the recovery.

MR. RODDY: We are giving full recognition to the work done by John Arthur Wilson, Hollander, and previous investigators.

Our major issue here, of course, is to show that it is possible by chemical analysis or a chemical technique to isolate the elastic tissue.

I appreciate, as you do, Dr. Turley, the work that has gone on before, not only by John Arthur Wilson, but also by Dr. Merrill who is in the audience, and who has used a similar technique for enzyme removal of the elastic tissue of calfskin, and I would like to go on record to say that is the case.

In regard to using the pure elastic tissue, some years back when we presented information on using the elastic tissue as a biological source from the neck ligament, it was argued as to whether it would resemble the elastic tissue in animal skin.

I might point out that in the course of doing this work in Cincinnati we had a man by the name of Earl Chia, from Formosa, visit our laboratory. Upon seeing the elastic tissue removed from skin and hide, and the ligament put into the autoclave for digestion purposes, he was very curious. I explained what we were doing. And of course he predates anyone else from the standpoint of the record of using a technique of this nature. He says that it was handed down in his family for generations. The pressure cooker was used to digest ligament and collagen to break it down to protein of a soluble nature and the right size so that it could be ingested by the family. So it predates everything.

As I say, then, Dr. Turley, I would like to give recognition to the gentlemen mentioned and to anyone else who has been concerned with this type of work.